

WHAT IS CLAIMED IS:

1. A method of detecting an extension reaction in which an extension reaction of a primer is detected, said method comprises the following steps of:

the step (a) of preparing a sample solution containing a nucleic acid, a primer having a base sequence that includes a complementary binding region which complementarily binds to said nucleic acid, and a nucleotide;

the step (b) of allowing said sample solution to stand under a condition to cause said extension reaction, and producing pyrophosphate when said extension reaction is caused;

the step (c) of bringing said sample solution into contact with the front face of a  $H^+$  hardly permeable membrane having  $H^+$ -pyrophosphatase, which penetrates from front to back of the membrane, of which active site that hydrolyzes pyrophosphate being exposed to the front face;

the step (d) of measuring the  $H^+$  concentration of at least either one of the solution at the front face side of said  $H^+$  hardly permeable membrane or the solution at the back face side of said  $H^+$  hardly permeable membrane, in a state where said  $H^+$ -pyrophosphatase is immersed in the solution; and

the step (e) of detecting said extension reaction on the basis of the result of measurement in the step (d).

2. A method of discriminating a base type in which the base type in a base sequence of a nucleic acid is discriminated, said method comprises the following steps of:

the step (a) of preparing a sample solution containing a nucleic acid, a primer having a base sequence that includes a complementary binding region which complementarily binds to said nucleic acid, and a nucleotide;

the step (b) of allowing said sample solution to stand under a condition to cause an extension reaction of said primer, and producing pyrophosphate when said extension reaction is caused;

the step (c) of bringing said sample solution into contact with the front face of a  $H^+$  hardly permeable membrane having  $H^+$ -pyrophosphatase, which penetrates from front to back of the membrane, of which active site that hydrolyzes pyrophosphate being exposed to the front face;

the step (d) of measuring the  $H^+$  concentration of at least either one of the solution at the front face side of said  $H^+$  hardly permeable membrane or the solution at the back face side of said  $H^+$  hardly permeable membrane, in a state where said  $H^+$ -pyrophosphatase is immersed in the solution;

the step (e) of detecting said extension reaction on the basis of the result of measurement in the step (d); and

the step (f) of discriminating the base type in the base sequence of said nucleic acid on the basis of the result of detection in the step (e).

3. The method of discriminating a base type according to claim 2 wherein the difference between the  $H^+$  concentration of the solution at said front face side, and the  $H^+$  concentration of said sample solution post the step (b) and before the step (c) is measured, in the step (d).

4. The method of discriminating a base type according to claim 3 wherein said extension reaction is detected by comparing the result of measurement in the step (d) with a control value, in the step (e).

5. The method of discriminating a base type according to claim 4 wherein said discrimination of a base type is the

discrimination of the base type of a SNP site, and  
said control value is the result of measurement obtained  
in the step (d) through carrying out the steps (a), (b), (c)  
and (d) using a nucleic acid having said SNP site without  
mutation, as said nucleic acid.

6. The method of discriminating a base type according  
to claim 2 wherein the  $H^+$  concentration of the solution at  
said back face side is detected in the step (d), and  
said extension reaction is detected by comparing the  
result of measurement in the step (d) with a control value,  
in the step (e).

7. The method of discriminating a base type according  
to claim 6 wherein said discrimination of a base type is the  
discrimination of the base type of a SNP site,

one kind of a nucleotide is used as said nucleotide in  
the step (a), and

said control value is the result of measurement obtained  
in the step (d) through carrying out the steps (a), (b), (c)  
and (d) using a nucleic acid having said SNP site with a  
different base type, as said nucleic acid.

8. The method of discriminating a base type according  
to claim 2 wherein said  $H^+$  concentration is optically measured  
in the step (d).

9. The method of discriminating a base type according  
to claim 8 wherein a pH sensitive pigment or a membrane  
potential sensitive pigment is added to at least either one  
of the solution at said front face side and the solution at  
the back face side, in the step (d).

10. The method of discriminating a base type according to claim 9 wherein acridine orange or Oxol V is added to at least either one of the solution at said front face side and the solution at the back face side, in the step (d).

11. The method of discriminating a base type according to claim 2 wherein said  $H^+$  concentration is electrically measured in the step (d).

12. The method of discriminating a base type according to claim 2 wherein said extension reaction is an extension reaction according to a PCR method.

13. A device for discriminating a base type in which the base type in a base sequence of a nucleic acid is discriminated which comprises:

a reaction section in which thermoregulation required for an extension reaction of a primer is carried out, and

a pyrophosphate detection section in which pyrophosphate that is produced by said primer extension reaction is detected,

wherein said reaction section is provided with a reserving region for reaction where a solution is reserved,

said pyrophosphate detection section is provided with a reserving region for detection where a solution is reserved, a  $H^+$  hardly permeable membrane that separates said reserving region for detection to a first region and a second region, and a measurement means for measuring the  $H^+$  concentration of the solution reserved in at least either one of the first region and second region, and

wherein said  $H^+$  hardly permeable membrane has  $H^+$ -pyrophosphatase, which penetrates from front to back of the membrane, of which active site that hydrolyzes pyrophosphate being exposed to the front face, and

in said pyrophosphate detection section, the reaction solution which is delivered from said reaction section is reserved in the first region.

14. The device for discriminating a base type according to claim 13 wherein said measurement means optically measures the  $H^+$  concentration.

15. The device for discriminating a base type according to claim 13 wherein said measurement means electrically measures the  $H^+$  concentration.

16. The device for discriminating a base type according to claim 13 further comprising an analysis means for controlling said reaction section and said pyrophosphate detection section, and for analyzing the result of measurement from said measurement means.

17. The device for discriminating a base type according to claim 13 further comprising a slot to which a tip can be inserted that is provided with said reserving region for reaction and said reserving region for detection.

18. A device for detecting pyrophosphate which comprises a vessel, a  $H^+$  hardly permeable membrane that separates inside of said vessel into a first region and a second region, an electrode that is provided such that it is brought into contact with a solution reserved in the first region, and a  $H^+$  sensitive electrode that is provided such that it is brought into contact with a solution reserved in the second region,

wherein said  $H^+$  hardly permeable membrane has  $H^+$ -pyrophosphatase, which penetrates from front to back of the membrane, of which active site that hydrolyzes

pyrophosphate being exposed to the front face.

19. A method of detecting a nucleic acid having a particular base sequence, said method comprises the following steps of:

the step (a) of preparing a sample solution containing a sample, a primer having a base sequence that includes a complementary binding region which complementarily binds to said nucleic acid, and a nucleotide;

the step (b) of allowing said sample solution to stand under a condition to cause an extension reaction of said primer, and producing pyrophosphate when said extension reaction is caused;

the step (c) of bringing said sample solution into contact with the front face of a  $H^+$  hardly permeable membrane having  $H^+$ -pyrophosphatase, which penetrates from front to back of the membrane, of which active site that hydrolyzes pyrophosphate being exposed to the front face;

the step (d) of measuring the  $H^+$  concentration of at least either one of the solution at the front face side of said  $H^+$  hardly permeable membrane or the solution at the back face side of said  $H^+$  hardly permeable membrane, in a state where said  $H^+$ -pyrophosphatase is immersed in the solution;

the step (e) of detecting said extension reaction on the basis of the result of measurement in the step (d); and

the step (f) of detecting the nucleic acid on the basis of the result of detection in the step (e).

20. The method of detecting a nucleic acid according to claim 19 wherein the difference between the  $H^+$  concentration of the solution at said front face side and the  $H^+$  concentration of said sample solution post the step (b) and before the step (c) is measured in the step (d).

21. The method of detecting a nucleic acid according to claim 20 wherein said extension reaction is detected by comparing the result of measurement in the step (d) with a control value, in the step (e).

22. The method of detecting a nucleic acid according to claim 21 wherein said control value is the result of measurement obtained in the step (d) through carrying out the steps (a), (b), (c) and (d) using said sample without including a nucleic acid.

23. The method of detecting a nucleic acid according to claim 19 wherein said  $H^+$  concentration is optically measured in the step (d).

24. The method of detecting a nucleic acid according to claim 23 wherein a pH sensitive pigment or a membrane potential sensitive pigment is added to at least either one of the solution at said front face side and the solution at said back face side, in the step (d).

25. The method of detecting a nucleic acid according to claim 24 wherein acridine orange or Oxol V is added to at least either one of the solution at said front face side and the solution at said back face side, in the step (d).

26. The method of detecting a nucleic acid according to claim 19 wherein said  $H^+$  concentration is electrically measured in the step (d).

27. The method of detecting a nucleic acid according to claim 19 wherein said extension reaction is an extension

reaction according to a PCR method.

28. A tip for introducing a sample solution which comprises a reaction chamber for carrying out an extension reaction of a primer, a pyrophosphate detection chamber for detecting pyrophosphate, and a flow pass that connects said reaction chamber and said pyrophosphate detection chamber.

29. The tip for introducing a sample solution according to claim 28 wherein said flow pass can be opened and closed.

30. The tip for introducing a sample solution according to claim 28 wherein said pyrophosphate detection chamber has a first region and a second region which are separated by a  $H^+$  hardly permeable membrane;

said  $H^+$  hardly permeable membrane has  $H^+$ -pyrophosphatase, which penetrates from front to back of the membrane, of which active site that hydrolyzes pyrophosphate being exposed to the front face; and

in said pyrophosphate detection chamber, the reaction solution that is delivered from said reaction chamber via said flow pass is reserved in the first region.